

Immobilized poly-L-histidine for chelation of metal cations and metal oxyanions

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Received 13 March 2003; received in revised form 19 August 2003; accepted 20 August 2003

Abstract

The biohomopolymer poly-L-histidine (PLHis) was immobilized onto controlled pore glass (CPG) and its metal binding capabilities evaluated through the use of a flow injection-flame atomic absorption system. The metal binding capability of PLHis-CPG was determined through the analysis of the generated breakthrough curves. The polymer likely coordinates cationic metals through the imidazole side chain ($pK_a \approx 6$) present on each histidine residue with both strong and weak binding sites for Cu^{2+} , Cd^{2+} , Co^{2+} , and Ni^{2+} . Weak to minimal binding was observed for Mn^{2+} , Ca^{2+} , Mg^{2+} , Na^+ , and Cr^{3+} . The bound metals are quantitatively released from the column with an acid strip. It has also been shown that the protonated imidazole side chain present in acidic solutions is capable of binding metal oxyanions such as chromates, arsenates, and selenites; although oxyanion binding currently exhibits interferences from competing anions in solution, such as sulfate and nitrate. The interference in oxyanion binding is less severe in the presence of chloride, phosphate, and acetate. PLHis-CPG exhibits a capacity of $\sim 30 \mu\text{mol Cu}^{2+}/\text{g CPG}$ in neutral to basic conditions, and a capacity of $\sim 70 \mu\text{mol Cr(VI)}/\text{g CPG}$, $\sim 4 \mu\text{mol As(V)}/\text{g CPG}$, and $\sim 4 \mu\text{mol Se(IV)}/\text{g CPG}$ in acidic conditions.

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Keywords: Poly-L-histidine; Controlled pore glass; Chromium; Chromate; Separation; Ion exchange; Flow injection analysis; Flame atomic absorption spectrometry

1. Introduction

In recent years, the investigation into methods for the removal of metals from the environment has been a major area of research due to the health risks they pose. A significant amount of research has focused on the development of novel ion-exchange systems for the remediation and preconcentration of metals from natural and industrial wastewaters. These exchangers can also be combined with flow injection analysis (FIA) and atomic spectrometry for preconcentration

and monitoring. A unique class of exchangers involves the use of immobilized short chain biohomopolymers (peptides) [1–7].

The ideal ion exchange system should allow for highly selective and specific chelation of target metals, offering strong binding of the metal of interest. The selectivity also optimizes the effective capacity of the exchanger because of the absence of competition for binding sites by other metals. It is important for the target metal to be easily released to enhance the preconcentration ratio for analytical applications or to minimize the effluent waste volume in remediation efforts. Additionally, quantitative release permits complete column regeneration. A chemically and mechanically stable support as well as irreversible

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attachment of the chelator to the support contributes to the cost effectiveness and lifetime of the system.

Previous work with immobilized biohomopolymers used for cation exchange has demonstrated that quantitative release can be achieved by simply lowering the pH of the column [2–5,8,9]. It was suggested [5] and later shown [10] that acid caused a reversible change in the tertiary structure to provide efficient and rapid release of the metal from the binding cavity. It was also demonstrated that these biohomopolymeric systems remain functional, with minimal loss of capacity, despite repeated exposure to harsh and varied chemical conditions over extended periods of time [5].

Sahni et al. [11] have shown the utility of imidazole or poly-imidazole derivatives in chelation systems. Additionally, Moreira and Gushikem used silica gel functionalized with 3(1-imidazolyl)propyl groups for preconcentration of Cu, Ni, Fe, Zn, and Cd from ethanol [12]. It is also well known that histidine is one of the primary metal binding sites in proteins and that the imidazole side chain is responsible for metal complexation [13–16]. In fact, a sequence of 4–6 histidines is frequently utilized as a “His-tag” in protein purification on a metal column in such techniques as immobilized metal ion affinity chromatography (IMAC).

The purpose of this study was to evaluate and characterize a poly-L-histidine (PLHis) ($n \sim 90$ –100) bonded phase ion exchange system for use in metal remediation and preconcentration, with particular emphasis on using the protonated imidazole in acidic media to bind oxyanions of Cr(VI), As(V), and Se(IV).

2. Experimental

2.1. Instrumentation

A Perkin-Elmer model 4000 atomic absorption spectrophotometer with an air/acetylene flame was used for all metal determinations. Hollow cathode lamps for the metals of interest were operated at the currents recommended by their manufacturers. Wavelengths for As, Ca, Cd, Co, Cr, Cu, Mg, Mn, Na, Ni, Pb, and Se were 193.7, 422.7, 228.8, 240.7, 357.9, 324.8, 285.2, 279.5, 589.0, 232.0, 283.3, and 196.0 nm, respectively. A monochromator band-pass of 0.2 nm was used for Co, Mn, and Ni, 0.4 nm for Na, 0.7 nm for As, Ca, Cd, Cr, Cu, Mg, Pb, and 2 nm for Se.

The simple flow injection manifold consisted of an eight-roller peristaltic pump (Ismatec minicartridge MS-REGLO) and two two-way, double inlet rotary valves (Rheodyne 5020). All connections were made with 0.76 mm i.d. PTFE tubing [3].

0.0727 g of immobilized PLHis-CPG was packed into a 3 mm i.d. \times 25 mm long glass column with 70 mm PTFE frits (Omnifit). A Kel-F tee was placed between the column and the nebulizer to provide air compensation and to minimize noise.

A Varian VGA-76 Vapor Generation Accessory was used in conjunction with the AA and the peristaltic pump for arsenic and selenium determinations. The vapor generation accessory was operated according to the manufacturer's instructions with the effluent from the column flowing into the sample line of the hydride vapor generator. Since the generator requires a flow of approximately 8 ml/min and the effluent from the column is only 1 ml/min, a tee was added to the sample line to mix 7 M HCl at a flow of 7 ml/min with the column effluent.

2.2. Reagents

All chemicals were reagent grade unless noted and deionized, distilled water was used to prepare solutions. All glassware was soaked in 4 M HNO₃ overnight before use. Poly-L-histidine (Sigma) [DP(LALLS) 104, MW(LALLS) 14,300] was used as received. The controlled pore glass (SIGMA, PG240-120) had a mean pore diameter of 22.6 nm and a mesh size of 80–120. Other reagents included 3-aminopropyltriethoxysilane (98%), nitric acid (Aldrich); acetic acid, hydrochloric acid, sodium phosphate (Fisher Scientific); ammonium acetate, ammonium hydroxide (Mallinckrodt); sodium acetate (Spectrum Chemical Mfg. Corp.); glutaraldehyde (25%) (Sigma); and sodium hydroxide and sodium borohydride (EM Science). Stock solutions of Ca²⁺ (Fisher); Cd²⁺, selenious acid (Inorganic Ventures); potassium chromate (Ricca Chemical Company); Cu²⁺, and Pb²⁺ (SCP Science) atomic absorption standards were used to prepare the 10 ppm loading solutions for the metal binding experiments. For Co²⁺, Na⁺, Ni²⁺ (Baker), Mg²⁺, and Mn²⁺ (Matheson, Coleman & Bell) the loading solutions were prepared from standardized solutions of the reagent grade nitrate salt. A 1000 ppm As stock solution was prepared

from sodium arsenate (Sigma) and diluted to provide the 500 ppb loading solution. 0.5 M ammonium acetate and 0.5 M sodium acetate stock solutions were prepared and purified using a 100–200 mesh Chelex 100 (Bio-Rad) ion exchange column.

2.3. Immobilization of PLHis onto CPG

Using a modification [4] of a procedure originally described by Masoom and Townshend [17], the CPG was activated by boiling ~1 g of the glass in 5% HNO₃ for 90 min. The acid activated CPG was filtered in a medium coarse, sintered glass filter, rinsed with distilled/DI H₂O, and dried in an 80 °C oven. An amine terminus was created on the surface of the glass through the use of 3-aminopropyltriethoxysilane (3-APS), a silanizing agent. The activated glass was reacted with 50 ml of 10% 3-APS, pH adjusted to pH 3.4 with HCl, at 75 °C for 150 min under nitrogen. After silanization, the glass was filtered in a medium coarse, sintered glass filter, rinsed with distilled/DI H₂O, and dried in an 80 °C oven. The silanization step was repeated a second time to ensure complete silanization of the glass, providing an amine terminus for linking with glutaraldehyde. Once the glass was silanized, rinsed and dried overnight at 80 °C, it was allowed to react with 50 ml of 5% glutaraldehyde in a 0.01 M phosphate buffer (pH 8.0) under nitrogen for 90 min at room temperature. The glutaraldehyde serves as a linker between the amine terminus on the 3-APS and the amine terminus of the poly-amino acid. Once the glutaraldehyde was attached, the glass was filtered and rinsed with distilled/DI H₂O. Twenty milligrams of PLHis, dissolved in 20 ml of pH 5.0, 0.01 M phosphate buffer was allowed to react with ~1 g of glutaraldehyde-CPG for 48 h at room temperature, under N₂. Upon completion of the immobilization the PLHis-CPG was rinsed, filtered, dried and packed in the microcolumn. The column required ca. 0.1 g of PLHis-CPG. The remainder of the PLHis-CPG was stored in a desiccator.

2.4. Binding of metal cations to PLHis-CPG

The previously described flow injection analysis system was utilized in all metal binding experiments. The pumps, tubing, hollow cathode lamp and flame

were warmed up for at least 15 min prior to use. After conditioning the column, unretained, acidified (pH <1.0) metal standards were run through the PLHis-CPG column and their absorbance values were used to prepare a calibration curve of the metal solution through the column.

Upon completion of the acid calibration curve, a 0.05 M ammonium acetate solution (pH 7.0) was pumped through the column for 2 min at 1 ml/min to recondition the column to the neutral pH and solution conditions. The cation metal binding solutions were prepared by dilution from the metal standards into 0.05 M ammonium acetate, diluted from the stock, and adjusted to pH 7.0 by drop wise addition of acetic acid or ammonium hydroxide. The 10 ppm ammonium acetate-metal solution was then introduced onto the column at a flow rate of 1 ml/min and the effluent concentration was detected by flame atomic absorption spectrometry producing a breakthrough curve. Once the effluent concentration equaled the influent concentration, the sample flow was stopped. Ammonium acetate was passed through the column and emptied into waste for ~10 s (i.e. 0.16 ml) to remove the remaining metal-containing solution from the lines and the column dead volume. The metals were stripped from the column by flowing 0.1 M HNO₃ for 5 min at 1 ml/min through the column and collecting the effluent in a 25 ml volumetric flask for subsequent analysis by FAA. Although it has been shown in previous studies that the metals are stripped from the column in only a few hundred microliters of acid [3], 5 ml was used to ensure complete removal of the metal from the column. The strip solution was not analyzed by direct transfer from the column to the FAA because the concentrations released far exceeded the dynamic range of the detection system. Breakthrough curves and strip solution data were analyzed for each of the target cations (Ca²⁺, Cd²⁺, Co²⁺, Cr³⁺, Cu²⁺, Mg²⁺, Mn²⁺, Na⁺, Ni²⁺, and Pb²⁺), resulting in the relative binding capacity of PLHis for each of the metals. All metal binding experiments were performed in triplicate.

A more detailed evaluation of the PLHis-CPG column capacity for Cu²⁺ was also undertaken for pH values ranging from 4.0 to 8.0 using a 10 ppm Cu²⁺ influent solution in 0.05 M ammonium acetate, pH adjusted with ammonium hydroxide or acetic acid, pumped through the column at a flow

rate of 1.0 ml/min. Breakthrough curves and strips were collected and evaluated using the metal binding procedure described previously.

2.5. pH-dependent binding of oxyanions of Cr(VI) to PLHis-CPG

The pH dependence of PLHis-CPG column capacity for Cr(VI) was determined under two sets of conditions. First, the Cr(VI) capacity was determined for pH values ranging from 1.0 to 6.0 using a 10 ppm Cr(VI) influent solution in 0.05 M ammonium acetate, pH adjusted with nitric acid. Second, the capacity was determined for pH values ranging from 3.0 to 6.0 with 10 ppm Cr(VI) in 0.05 M ammonium acetate, pH adjusted with acetic acid. All solutions were pumped through the column at a flow rate of 1.0 ml/min. Breakthrough curves were collected and evaluated using the procedure outline above.

2.6. Anion influence on Cr(VI) binding to PLHis-CPG under acidic conditions

In order to determine the impact of competing anions, Cr(VI) was run through the column using different acids. The FIA-FAA set-up and conditioning were the same as previously described. Prior to running the sample solution through the column for the breakthrough curve, the column was conditioned for 2 min with the metal-free acid solution that was about to be run. These solutions were: 0.1 M H₂SO₄, 0.01 M H₂SO₄, 0.1 M HNO₃, 0.01 M HNO₃, 0.1 M HCl, 0.01 M HCl, 0.1 M H₃PO₄, 0.01 M H₃PO₄, 1 M CH₃COOH, 0.1 M CH₃COOH, and 0.05 M CH₃COOH. In addition, Cr(VI) was also run in 0.5 M ammonium acetate and 0.5 M sodium acetate, both pH adjusted to pH 4.0 with acetic acid, to determine the effects of different cations in solution.

After conditioning the column with a metal-free solution of the acid to be evaluated, a 10 ppm Cr(VI) solution in the respective acid was pumped through the column and breakthrough data recorded. Once breakthrough was achieved the sample flow was stopped and 0.1 M HNO₃ was passed through the column and emptied into waste for ~10 s in order to remove the remaining metal-containing solution from the lines and the column dead volume. The Cr(VI) was stripped from the column with a flow of 0.1 M HNO₃ for 10 min at

1 ml/min. The strip solution was collected into a 25 ml volumetric flask for subsequent analysis.

2.7. Cr binding studies on modified surfaces of CPG

To confirm that the binding observed was due to the PLHis and not unreacted surface functionalities generated during the immobilization, the uptake of Cr(VI) and Cr³⁺, each in 0.1 M HNO₃ and in 0.05 M ammonium acetate (adjusted to pH 6.0), was evaluated separately on three modified CPG columns: 0.0704 g of acid activated CPG, 0.0757 g of silanized CPG and 0.0653 g of glutaraldehyde CPG. The metal influent concentration was 10 ppm and the influent flow rate was 1.0 ml/min. The loading and stripping procedure was the same as that described previously.

2.8. Binding of As(V) to PLHis-CPG

In order to determine if PLHis-CPG was able to bind arsenic, the hydride generator was positioned between the column and FAA. The FIA system, and the hydride generator were run as previously described, flowing either acid or sample through the column. After conditioning the column with 0.1 M HCl for 2 min, a 500 ppb As(V) solution in 0.1 M HCl was pumped through the column and breakthrough data were collected. Once breakthrough was achieved, 0.1 M HNO₃ was used to clear lines and strip the column as described above. To study the effects of various solution conditions on As(V) binding, 500 ppb of As(V) in a variety of solutions were run through the PLHis column and breakthrough data was collected. These solutions include: 0.05 M ammonium acetate at pH values of 4.0, 5.0, 6.0, and 7.0, using sodium hydroxide or acetic acid to adjust the pH, distilled/deionized H₂O, 0.1 M NaCl, 0.1 M HNO₃, 0.05 M Tris, 0.05 M phosphate buffer, 0.1 M HCl, 0.01 M HCl, 1.0 mM HCl, 0.1 mM HCl, 0.1 M HClO₄, 0.01 M HClO₄ and 1.0 mM HClO₄.

2.9. Binding of Se(IV) to PLHis-CPG

The Varian VGA-76 Vapor Generation Accessory was also used in all Se determinations. The generator was positioned and run in the same manner previously described for As(V) determinations. 500 ppb

Se(IV) was run on the PLHis-CPG column in various solutions: 0.1 M HCl, 0.05 M ammonium acetate, and distilled/deionized H₂O. Column conditioning and stripping used the protocol employed for the As(V) study.

3. Results and discussion

3.1. Metal binding characteristics of PLHis-CPG

Breakthrough curves run on the PLHis-CPG column were analyzed by monitoring the metal concentration in the column effluent by FIAS-FAA as a function of influent volume. Fig. 1 contains typical breakthrough curves for various metals. The flat baseline region present on the Cd²⁺ and Cu²⁺ curves generally represents strong metal binding sites ($\log K_{\text{effective}} > 8$). The sloped regions represent the weaker metal binding sites. The rapid rise of the effluent Na⁺ concentration to that of the influent is an indication of little or no binding capacity for this cation. Breakthrough analysis of Co²⁺ and Ni²⁺ showed both strong and weak binding sites, while Pb²⁺ demonstrated only weak sites. PLHis-CPG had very little, to no binding of Ca²⁺, Mn²⁺, Mg²⁺, Na⁺, and Cr³⁺. By integrating the breakthrough curve, the total amount of metal retained on the column can be determined. These values are validated using the results from the stripped solutions. Table 1 contains

Table 1

Cationic metal binding capacity on PLHis-CPG column

Metal ion	Breakthrough data ($\mu\text{mol/g CPG}$)	Strip data ($\mu\text{mol/g CPG}$)
Cu ²⁺	32.2 \pm 0.3	36.7 \pm 1.0
Cd ²⁺	8.4 \pm 1.9	8.5 \pm 0.4
Ni ²⁺	7.7 \pm 0.4	8.8 \pm 0.3
Co ²⁺	4.3 \pm 1.3	4.9 \pm 0.4
Pb ²⁺	1.7 \pm 0.2	2.4 \pm 0.6
Na ⁺	<1	0.6 \pm 0.2
Ca ²⁺	<1	0.5 \pm 0.1
Mg ²⁺	<1	0.3 \pm 0.2
Cr ³⁺	<1	0.20 \pm 0.02
Mn ²⁺	<1	0.14 \pm 0.04

Uncertainties, expressed as sample standard deviations, reflect measurement uncertainties only. pH: 7.0, flow rate: 1 ml/min, triplicate measurements.

a summary of the cationic metal binding results for PLHis-CPG. Although the difference in capacities calculated from the breakthrough and the strip are not large, several are bigger than would be expected based on the standard deviation for triplicate analysis. In some instances the differences are slightly greater than expected from measurement uncertainties. To ensure the excess metal in the strip solutions could not be attributed to contamination in the buffer, blank ammonium acetate solutions were analyzed by ICPMS before and after treatment with chelex-100. The Cu²⁺, Cd²⁺, Ni²⁺, and Pb²⁺ concentrations in ammonium acetate were all below the detection limit by ICPMS, i.e. less than 0.4, 0.2, 1.0, and 0.4 ppb,

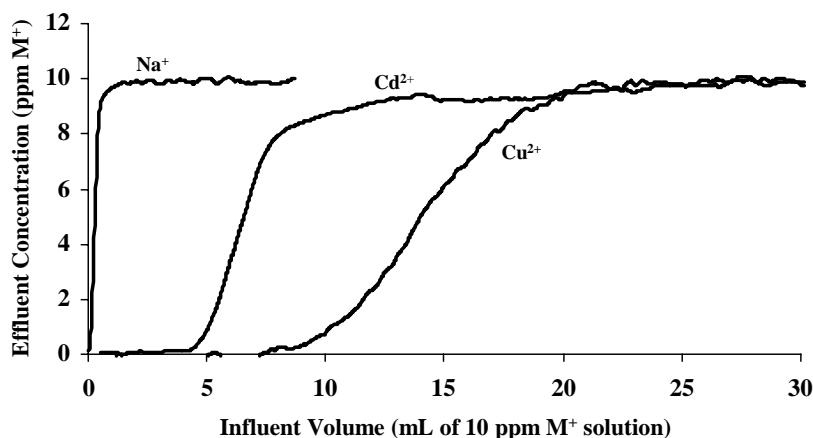


Fig. 1. Breakthrough curves of Na⁺, Cd²⁺, and Cu²⁺ on PLHis-CPG; demonstrating examples of minimal binding, moderate binding, and strong binding, respectively. All solutions were 10 ppm in the respective metal and 0.05 M ammonium acetate; pH 7.

respectively. Therefore, it must be concluded that the mass difference is not due to contamination, but may be due to systematic errors in analyzing the breakthrough data and/or collecting the strip solutions. More importantly, the relative binding trend is consistent between the breakthrough and the strip data. As a result, future comparisons of capacities are based on the breakthrough capacity values. The metal binding trend of PLHis-CPG was determined to be: $\text{Cu}^{2+} \gg \text{Cd}^{2+} \approx \text{Ni}^{2+} > \text{Co}^{2+} > \text{Pb}^{2+} \gg \text{Na}^+ \approx \text{Ca}^{2+} \approx \text{Mg}^{2+} \approx \text{Cr}^{3+} \approx \text{Mn}^{2+}$. This trend is in general agreement with that reported for metal binding to nitrogen ligands such as pyridine and imidazole [18].

The main metal *cationic* binding functionality of PLHis-CPG is the imidazole ring on each histidine residue. More specifically, the lone pair of electrons on the pyridine nitrogen of the imidazole ring is active in the coordination of metals to PLHis. McCurdie and Belfiore have demonstrated that PLHis forms solid state complexes, through the imidazole side chain, with the divalent metal chlorides of cobalt, nickel, copper, and zinc [19].

3.2. Binding of Cu^{2+} to PLHis-CPG with varying pH

The effect of pH on the capacity of the PLHis-CPG column for Cu^{2+} was determined for pH values

Table 2

Cu^{2+} binding capacity dependence on pH

pH	Breakthrough data ($\mu\text{mol/g}$ CPG)	Strip data ($\mu\text{mol/g}$ CPG)
3	2.28 ± 0.01	1.82 ± 0.07
4	10.5 ± 1.1	11.9 ± 0.3
5	18.1 ± 0.1	18.24 ± 0.04
6	22.5 ± 1.0	26.5 ± 0.2
7	32.2 ± 0.3	36.7 ± 1.0
8	36.4 ± 1.8	43 ± 2

10 ppm Cu^{2+} in 0.05 M ammonium acetate influent, flow rate: 1 ml/min, triplicate measurements.

4.0–8.0. The breakthrough curve variations with pH can be seen in Fig. 2 and the capacity values presented in Table 2. Present on each curve is a flat baseline region representative of strong binding sites and a sloped, weaker binding region. As the pH increases, there is a gain in both strong and weak site capacities.

Patchornik et al. estimated the pK_a of PLHis to be 6.15 [20], which is consistent with the reported values of 5.6–7.0 for the histidine residues in proteins [21] and very close to the value of 6.0 given for the imidazole side chain of histidine. However, it is important to note that it is reasonable to think of a broad range of pK_a s along the chain of the polymer and that the pK_a of 6.15 is simply a reasonable estimate. In fact, the pK_a may vary along the length of the chain with the surface as well as neighboring functional groups

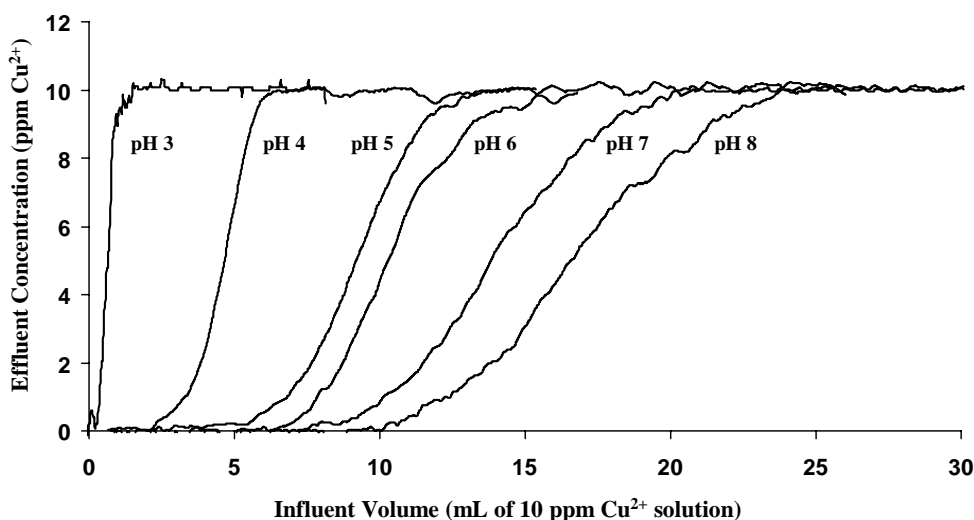


Fig. 2. Breakthrough curves of 10 ppm Cu^{2+} on PLHis-CPG in 0.05 M ammonium acetate at various pHs.

influencing local pK_a s. At pHs greater than this, the pyridine nitrogen on the imidazole side chain is deprotonated and has an unshared lone pair of electrons capable of metal binding. Measurable binding appears between pH 3 and 4, which is consistent with studies suggesting that the PLHis–Cu²⁺ complex begins to form at pH 3.0 [22–24]. This likely reflects the relative strength of the PLHis–Cu²⁺ coordination and its ability to displace the proton on the imidazole.

The data in Table 2 also indicate that there is a significant increase in metal binding capacity as the pH increases and as the percentage of deprotonated imidazoles and associated availability of lone pair electrons increases. It is interesting to note that the extent of PLHis protonation changes by ~3 orders of magnitude as the pH is lowered from 6.0 to 3.0, but the extent of Cu²⁺ binding only decreases by a factor of 10. This suggests that there must be some means of enhancing binding that offsets the significant loss of deprotonated imidazole binding sites. It is likely that a conformational change of this tethered, short-chain peptide at low pHs partially compensates for this decrease in deprotonated imidazole binding sites.

Previous studies have shown that PLHis, in solution, undergoes conformational changes with changes in pH [24–29]. It is generally agreed that a conformational transition occurs as the degree of protonation of the imidazole side chain changes, but there are differing opinions on the exact conformation of the chain, as it becomes deprotonated. Most recently, it has been concluded that at pH <4, where the imidazole is protonated, the chain exists as a random coil; and as the imidazole deprotonates, the structure moves toward a more ordered structure, resulting in a beta-pleated sheet at pH >5.2 [28]. Palumbo et al. studied the effects of the PLHis conformation on Cu²⁺ complexation and determined that when the chain exists as a random coil, the complexation occurs through three imidazole nitrogens and one peptide nitrogen in the square planar configuration (complex I). In contrast, if the chain is in a more ordered conformation, such as a beta sheet, the complexation occurs through four imidazole nitrogen in the square planar configuration (complex II) [24]. They also concluded that the Cu²⁺:peptide molar ratio had an effect on which complex formed, with complex I dominating at higher metal concentrations. Therefore, the immobilized PLHis most likely forms complex I, exclusively, at low pHs and as the pH is

raised it forms complex II at low Cu²⁺:peptide ratios. As the Cu²⁺ concentration increases, the beta sheet conformation gets disrupted and the coordination becomes that of complex I. More importantly, as both of these complexes form, the imidazole nitrogen remains as the primary binding functionality.

3.3. Binding of Cr(VI) to PLHis-CPG with varying pH

The presence of a positive, protonated imidazole under acidic conditions suggests the possible utility of PLHis for complexation of anions. More specifically, it may be useful as a chelator for metals that exist as oxyanions, such as the chromates, arsenates, etc. The pH dependence of the PLHis-CPG capacity for Cr(VI) was studied using two different means of adjusting the pH. All solutions were prepared in 0.05 M ammonium acetate, but one set was pH lowered with nitric acid the other set adjusted by acetic acid addition. Fig. 3 shows that pH had very little effect on the binding capacity or shape of the breakthrough curves when nitric acid was used to alter the pH. Additionally, the ionic strength of the solution pH adjusted with nitric acid is increasing as more acid is added to decrease the pH, and there is no significant change in capacity.

In contrast, Fig. 4 shows that pH had a significant effect on the binding capacity when the nitrate was omitted from the solution and the pH adjusted using acetic acid. The calculated capacities are listed in Table 3. The acetic acid data show a significant increase in the capacity as the pH drops from 6.0 to 5.0 and another increase as the pH drops from 5.0 to

Table 3
Cr(VI) binding capacity dependence on pH

pH	Cr(VI) bound nitric acid (μmol/g CPG)	Cr(VI) bound acetic acid (μmol/g CPG) ^a
1	10.1 ± 0.5	–
2	11.7 ± 1.0	–
3	8.7 ± 0.6	25.8 ± 0.8
4	7.9 ± 0.2	23.5 ± 1.1
5	8.1 ± 0.7	16.1 ± 0.3
6	10.0 ± 1.1	10.0 ± 1.1

10 ppm Cr(VI) in 0.05 M ammonium acetate influent, flow rate: 1 ml/min, triplicate measurements.

^a pH values <3 were unattainable using only acetic acid to adjust the pH.

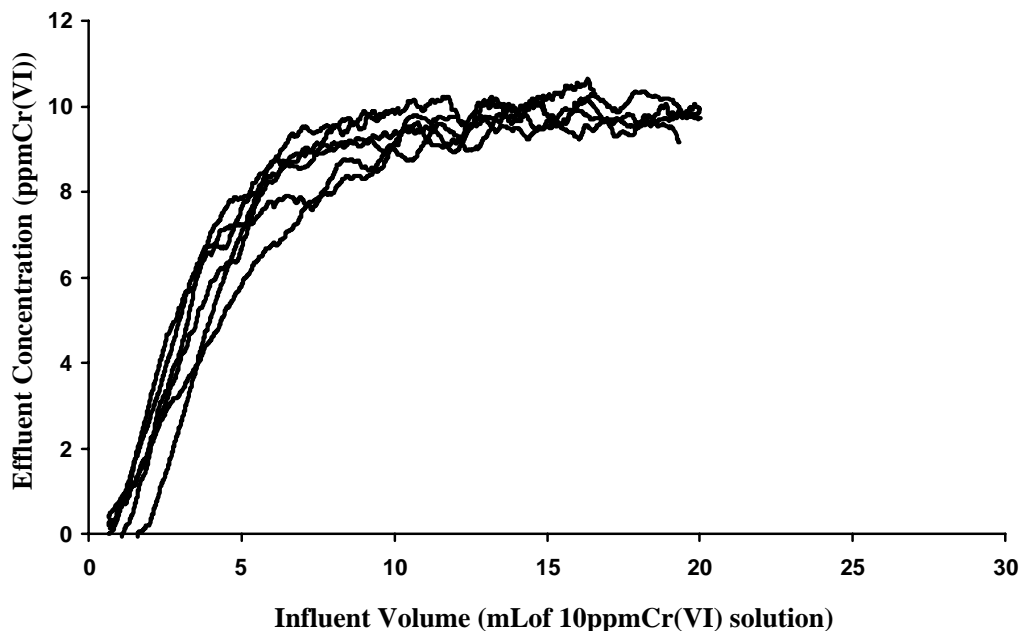


Fig. 3. Breakthrough curves of 10 ppm Cr(VI) on PLHis-CPG in 0.05 M ammonium acetate at various pHs. pH was adjusted with nitric acid.

4.0. This is consistent with a pK_a for the imidazole of PLHis of ~ 6.0 and conformation change from a beta sheet to a random coil at $pH < 5.2$ [28]. The column capacity for *anions* would be expected to rise

as the percentage of protonated imidazoles significantly increases (i.e. $pH < pK_a \sim 6.0$). In this pH range (3.0–6.0), the distribution of chromium species remains fairly constant with the predominant species

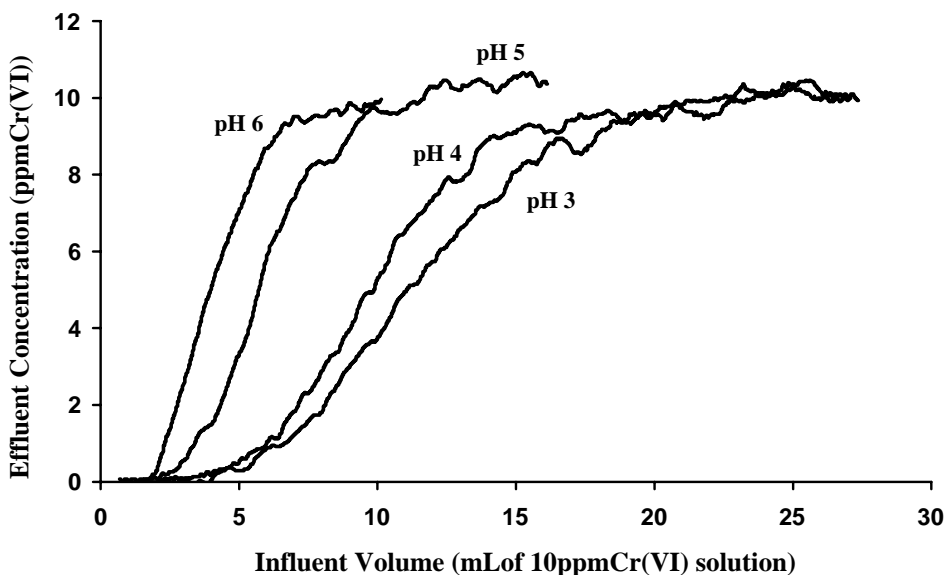


Fig. 4. Breakthrough curves of 10 ppm Cr(VI) on PLHis-CPG in 0.05 M ammonium acetate at various pHs. pH was adjusted with acetic acid.

being HCrO_4^- . Therefore, the change in capacity is likely the result of changes in the chelator rather than a change in the dominant chromium species present. The calculated capacities in Table 3 also suggest that the presence of nitrate in the solution inhibited Cr(VI) binding at all pHs. The strong influence shown by nitrate raised the concern that other anions may also significantly impact Cr(VI) binding.

3.4. Binding of Cr(VI) to PLHis-CPG in the presence of various anions

Since variations in the ion selectivity of ion exchangers may be caused by factors such as pH, ionic strength, competing background ligands and the nature of the exchanger [30]; the effects of CH_3COO^- , PO_4^{3-} , Cl^- , NO_3^- , and SO_4^{2-} , on PLHis-CPG, were evaluated by running Cr(VI) breakthrough curves in separate solutions to which CH_3COOH , H_3PO_4 , HCl , HNO_3 , and H_2SO_4 were added (Fig. 5). The Cr(VI) capacities calculated from these curves (Table 4) show that PLHis-CPG has the greatest capacity for Cr(VI) in acetic acid. It is evident from these curves that the other ions in solution (i.e. chloride, nitrate and sulfate) interfere with the binding. Also, as the concentration of the anions increased, the capacity for Cr(VI) decreased. While this appears to indicate that ionic strength may affect column capacity, a closer look at

Table 4

Cr(VI) binding capacity in various acidic solutions

Solution	pH	Cr(VI) bound ($\mu\text{mol/g CPG}$)
1.0 M CH_3COOH	2.9	66.2 ± 0.7
0.1 M CH_3COOH	3.8	75.5 ± 2.1
0.05 M CH_3COOH	3.9	77.2 ± 3.1
0.1 M H_3PO_4	2.1	34.8 ± 0.6
0.01 M H_3PO_4	3.0	53.8 ± 1.8
0.1 M HCl	1.0	20.3 ± 1.0
0.01 M HCl	2.4	46.6 ± 1.9
0.1 M HNO_3	1.0	9.6 ± 0.4
0.01 M HNO_3	2.3	40.7 ± 3.0
0.1 M H_2SO_4	1.4	9.8 ± 2.4
0.01 M H_2SO_4	2.3	10.1 ± 0.7

10 ppm Cr(VI) influent, flow rate: 1 ml/min, triplicate measurements.

the data suggests that the impact on binding is ion specific. For example, the ionic strength of 0.1 M HNO_3 is the same as the ionic strength of 0.1 M HCl , but HNO_3 affects the capacities much more significantly. A similar affect is seen for the same acids present at 0.01 M. In summary, the presence of sulfate ions caused the greatest loss in capacity followed by nitrate, chloride and phosphate.

These interferences are consistent with trends reported by Gang et al. [31] for Cr(VI) binding by poly(4-vinylpyridine) coated silica gel. Poly(4-vinylpyridine) coated silica gel showed a reduction

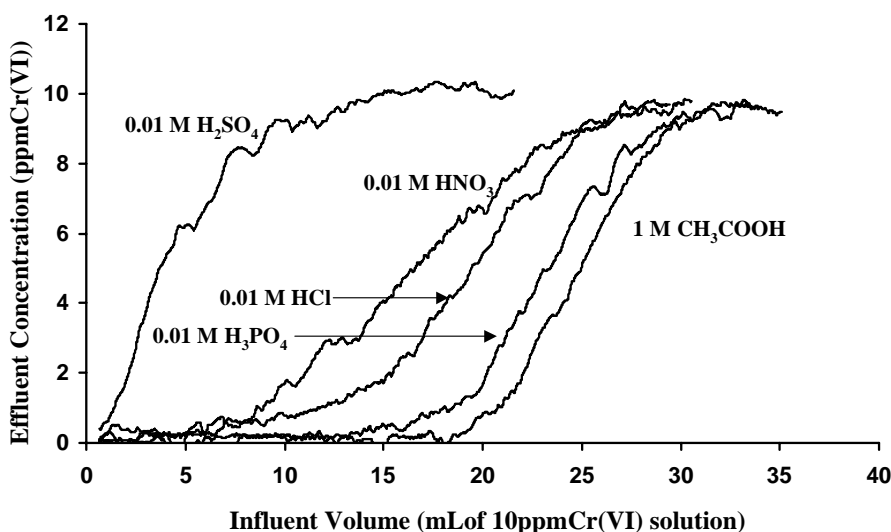


Fig. 5. Breakthrough curves of 10 ppm Cr(VI) on PLHis-CPG in solutions containing various acids.

in Cr(VI) capacity in the presence of Cl^- , SO_4^{2-} , and CH_3COO^- , with sulfate having the biggest impact followed by chloride and acetate. They also noted that competing anions had very little effect on capacity as long as their concentrations were less than or equal to the Cr(VI) concentration. At sulfate concentrations 250 times greater than the Cr(VI) concentration, a 50% decrease in capacity was seen [31].

Sengupta and Clifford performed extensive studies on the effects of sulfate and chloride on the removal of Cr(VI) with the styrene-divinylbenzene (STY-DVB) anion exchange resins (IRA-900 and IRA-94) and concluded that increasing the sulfate concentration increased the selectivity of the resin for Cr(VI) while an increase in the chloride concentration had no effect on the resin's selectivity for Cr(VI) [32]. Clifford also notes that there is only a slight reduction of Cr(VI) anion exchange capacity in the presence of Cl^- , and SO_4^{2-} on the commercially available strong base anions exchangers: IRA-900, Dowex 11, and IRA 958 [33].

Additionally, the effects of competing anions on Cr(VI) capacity was studied on a new, polymeric ligand anion exchanger (DOW 3N-Cu) by Zhao et al. [34]. This exchanger was able to effectively remove Cr(VI) from a background of competing anions such as sulfate, chloride, bicarbonate and nitrate, which were present at concentrations much higher than Cr(VI). This exchanger was capable of a much greater capacity for Cr(VI) than IRA-900, which was used for comparison in their study. Although the effects of various concentrations of competing anions in solution was not studied, breakthrough curves of HCO_3^- , NO_3^- , Cl^- , and SO_4^{2-} showed that each of these ions breakthrough well before Cr(VI) [34]. The *weak base* (imidazole) exchange sites of the PLHis are more susceptible to the influence of the tested anions than any of the other exchangers noted.

In order to take a closer look at the effect the ionic strength of the solution had on Cr(VI) binding, breakthrough analysis was conducted with 10 ppm Cr(VI) solutions in different concentrations of acetic acid (1.0, 0.1, and 0.05 M). The calculated capacities are listed at the top of Table 4. In each of these solutions the ionic strength will change with the concentration of acetic acid due to the extent of dissociation of acetic acid into acetate ions. The capacities in 0.1 and 0.05 M are the same and there is only a slight drop in capacity in

1.0 M acetic acid. It can be concluded that pH is not a factor in these three solutions because the pH only changes from 2.0 in 1.0 M acetic acid to 3.9 in 0.05 M acetic acid. In this range the chromium distribution remains constant with HCrO_4^- still dominating, and with a $\text{p}K_a$ of ~ 6.0 for the imidazole on the PLHis, at $\text{pH} < 4$ the imidazoles are $>99.9\%$ protonated. It is important to note that the Cr(VI) capacities in acetic acid are much greater than those calculated in ammonium acetate for all pH's studied. It is not obvious, however, if the presence of ammonium or the higher ionic strength is responsible for the decrease in capacity in the ammonium acetate solutions. Overall, the data indicate that the type of anion has the strongest effect on the Cr(VI) capacity. The ionic strength may play a role but its importance seems to be secondary in importance.

Upon completion of the anion studies Cu^{2+} , in ammonium acetate at pH 7, was run on the column to verify that the anions had not irreversibly altered the column.

The PLHis-CPG results show that although it has a significant capacity for Cr(VI) under certain conditions, it does have an affinity for other anions. Cr(VI) remediation by this column may be less effective if the samples contain significant concentrations of sulfates or nitrates. However, it is apparent from the long, flat baseline seen in the breakthrough curves of Cr(VI) in the presence of chloride, phosphate or acetate in Fig. 5 that this polymer could serve as an effective clean-up or polishing step in Cr(VI) removal.

3.5. Cr binding studies on modified surfaces of CPG

The immobilization procedure probably does not result in complete coverage of the CPG surface, and it has been shown previously that alkoxysilanes immobilized onto CPG are useful for oxyanion removal [35]. To evaluate the impact of non-PLHis surface binding, Cr(VI) capacities of the modified CPG were determined at each step of the immobilization: acid activated-CPG, silanized-CPG (amine terminated), gluteraldehyde-CPG, and PLHis-CPG. The breakthrough capacities for the metal solutions on these four different columns are listed in Table 5. These results are consistent with the expectation of significant Cr(VI) anion interactions with positively charged

Table 5
Cr binding capacity on CPG system at each stage of PLHis immobilization

Metal solution	Capacity ($\mu\text{mol/CPG}$)			
	Acid activated CPG	Silanized CPG	Gluteraldehyde CPG	PLHis-CPG
Cr(VI) acid	0.39 ± 0.07	8.3 ± 0.5	5.6 ± 0.1	18.6 ± 0.6
Cr(VI) neutral	0.23 ± 0.05	2.5 ± 0.2	0.7 ± 0.1	2.3 ± 0.2
Cr ³⁺ acid	0.20 ± 0.06	0.33 ± 0.04	0.19 ± 0.01	0.28 ± 0.01
Cr ³⁺ neutral	0.36 ± 0.09	1.2 ± 0.3	0.5 ± 0.2	0.35 ± 0.07

10 ppm Cr influent, flow rate: 1 ml/min, triplicate measurements.

species on the surface of the CPG, which are only present after the silanization step and the PLHis immobilization. In fact, capacity is gained with the addition of the silanizing agent after the acid activation but lost when gluteraldehyde is attached, blocking the amine terminus that was present for binding after the silanization step. The binding capacity increased again with the immobilization of the PLHis to the gluteraldehyde. This trend indicates that the metal binding capabilities of the PLHis-CPG column is due to the PLHis chelator with very little contribution from the unblocked activated glass surface, amine groups on the silanizing agent and gluteraldehyde groups. The capacities for each of these surfaces were calculated from breakthrough curves of Cr(VI) in nitric acid and in ammonium acetate at pH 7. In contrast to Cr(VI), Table 5 shows that each stage of the immobilization exhibited very little capacity for Cr³⁺ in either nitric acid or ammonium acetate.

3.6. Binding of As(V) to PLHis-CPG

Similar to Cr(VI), arsenic exists as an oxyanion in solution and may effectively bind to the PLHis column. Using As(V) as the target binding species, the sensitivity of the hydride generation FAAS (HG-FAAS) detection permitted the use of a 500 ppb As(V) influent solution. The only solution that showed significant binding was As(V) in distilled/deionized water and As(V) in 0.1 mM HCl. The calculated capacity was determined to be $4.5 \pm 0.5 \mu\text{mol As/g PLHis-CPG}$. Several representative breakthrough curves of As(V) in various concentrations of HCl can be seen in Fig. 6. While the disruption of binding showed interferences similar to that seen for Cr(VI), they were much more severe in the case of As(V). Previous studies that demonstrated an anion exchange capacity of 106 mg As/g dry resin (poly(ethylenmercaptopoacemide)) at pH 2, also experienced a reduction in capacity in the

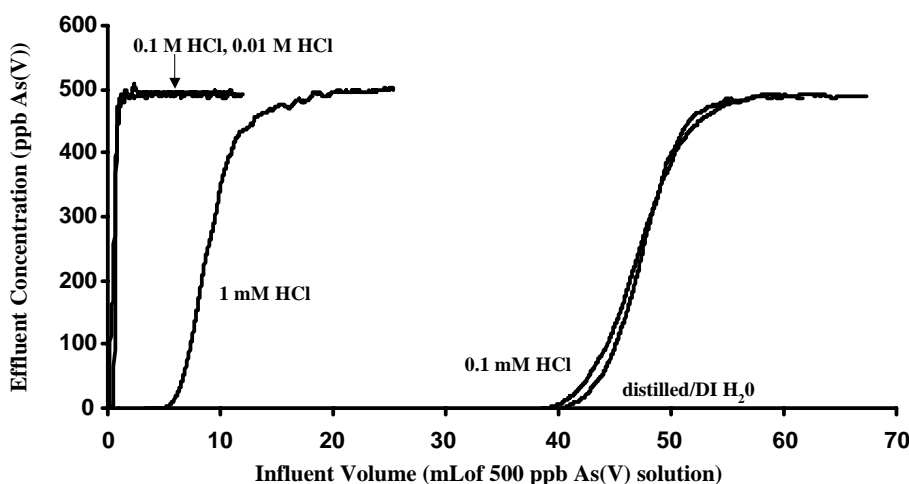


Fig. 6. Breakthrough curves of 500 ppb As(V) on PLHis-CPG in various solutions.

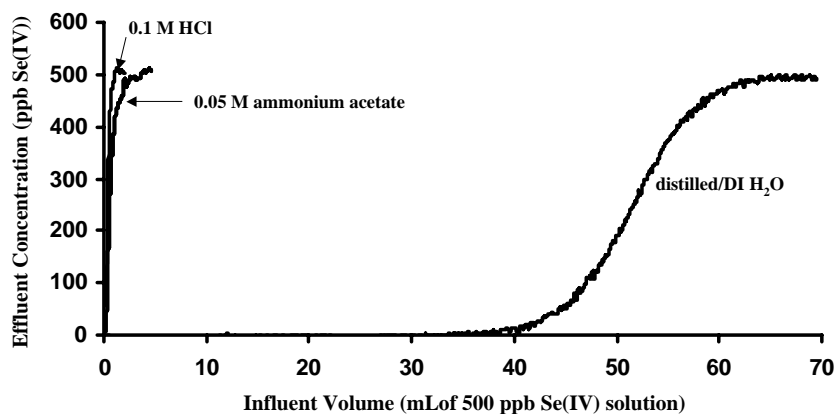


Fig. 7. Breakthrough curves of 500 ppb Se(IV) on PLHis-CPG in various solutions.

presence of other anions (Cl^- and SO_4^{2-}) [36]. It has also been shown that the As(V) capacity is very significantly reduced on the commercially available exchange resin, Dowex 11, in the presence of SO_4^{2-} [33].

Although the capacity of PLHis-CPG for As(V) is not very large, $4.5 \pm 0.5 \mu\text{mol As/g CPG}$; the extended baseline in the breakthrough curves indicates a dominance of strong binding sites ($K > 10^6$) with effluent concentrations below 8 ppb, the limit of detection for the HG-FAAS system.

3.7. Binding of Se(IV) to PLHis-CPG

Fig. 7 shows several Se(IV) breakthrough curves. As these curves indicate, Se(IV) was only retained on the PLHis-CPG column in distilled/DI H_2O . In each of the solutions tested it behaved similarly to As(V). The maximum capacity for Se(IV), in distilled/DI H_2O , was calculated to be $4.6 \pm 0.4 \mu\text{mol Se/g CPG}$. Clearly, Se(IV) suffers from the same anion interferences as As(V).

It is important to note that while the cation metals are easily stripped from the PLHis-CPG column in a few hundred microliters of acid, the oxyanions do not come off as easily. Attempts were made to strip the oxyanions off the column using a variety of solutions. The most effective means of stripping the oxyanions was determined to be 0.1 M HNO_3 , the same solution used to remove chelated cations from PLHis-CPG. Each of the oxyanions studied can be quantitatively

removed from the column in 10 ml of 0.1 M HNO_3 flowing at 1 ml/min. As a result, PLHis-CPG may not be an effective means of metal oxyanion preconcentration.

4. Conclusions

It has been shown that PLHis-CPG is an effective metal chelator. The metal cation binding trend is $\text{Cu}^{2+} \gg \text{Cd}^{2+} \approx \text{Ni}^{2+} > \text{Co}^{2+} > \text{Pb}^{2+} \gg \text{Na}^+ \approx \text{Ca}^{2+} \approx \text{Mg}^{2+} \approx \text{Cr}^{3+} \approx \text{Mn}^{2+}$, with a capacity for Cu^{2+} calculated from breakthrough analysis of $32.2 \pm 0.3 \mu\text{mol Cu}^{2+}/\text{g CPG}$. Each of the metal cations can be quantitatively stripped from the column in several hundred microliters of 0.1 M HNO_3 . The pH study conducted with metal cations showed an increase in capacity with increasing pH, most likely due to conformational changes of the polymer. PLHis-CPG was also shown to bind the oxyanions of Cr(VI), As(V) and Se(IV) in acidic solution through the protonated imidazole. Oxyanion binding with PLHis-CPG suffers from a reduced capacity in the presence of competing anions in solution with interferences decreasing in the order: sulfate, nitrate, chloride, phosphate, and acetate. The imidazole side chains of PLHis are weak base exchanger sites, which limits their selectivity for the metal-oxyanions investigated. Due to the fact that the oxyanions are not as easily released, PLHis-CPG is not a likely candidate for oxyanion preconcentration. However, the efficient binding suggests utility as

a polishing step in oxyanion remediation although a more efficient means of column reclamation is still needed to minimize the volume of the strip solution.

Acknowledgements

This work was supported, in part, by the Texas Hazardous Waste Research Center and the Robert A. Welch Foundation. We would also like to thank Dr. Karen Browning for her contributions to this study.

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